SYNTHESIS OF STEROIDS CONTAINING THE α-KETO ACID SIDE-CHAIN (1a)

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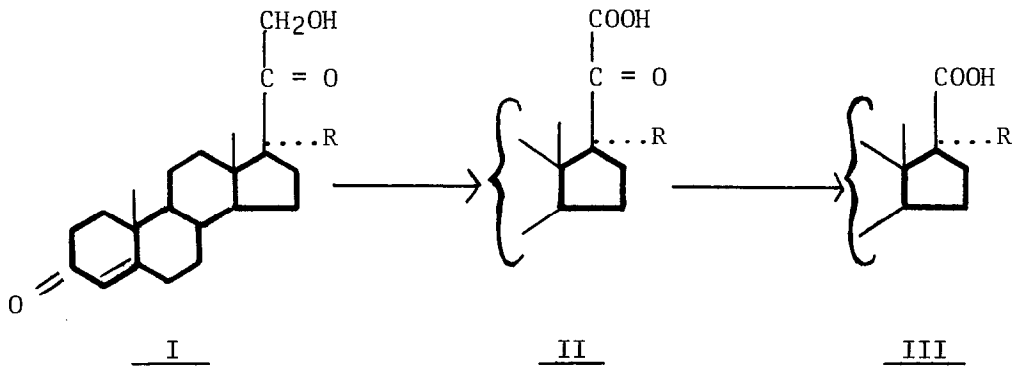
ABSTRACT

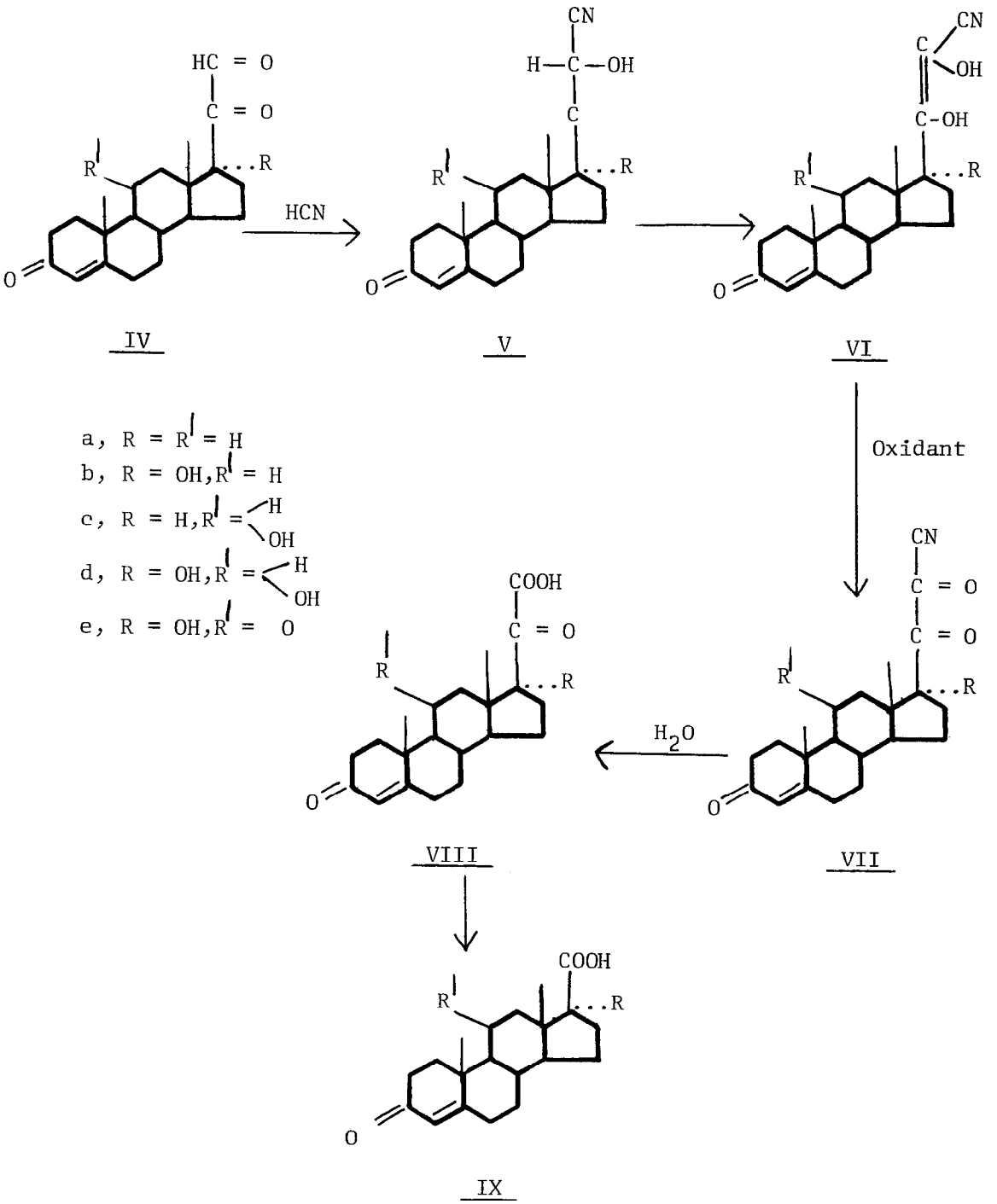
A general method for the chemical synthesis of steroidal 20-keto-21-oic acids is described. 21-Dehydrocorticosteroids, in the presence of catalytic amounts of cyanide ion, are oxidized by methylene blue or chromium trioxide at neutral pH to the corresponding keto acids.

Mammalian tissues oxidize the 21-hydroxy-20-keto side-chains of corticosteroids I to 17β-carboxylic acids III with the attendant loss of one carbon atom (2-5). It has been suggested by Schneider (6) that this conversion takes place by the oxidative decarboxylation of an α-keto acid intermediate II:



I II III

He obtained 3,20-diketo-4-pregnene-21-oic acid II, R = H, both by incubation of 21-hydroxy-4-pregnene-3,20-dione with guinea pig liver and by oxidation of 3,20-diketo-4-pregnene-21-al with alkaline silver nitrate (6). This latter method cannot be applied to steroids containing the dihydroxyacetone side-chain since alkaline silver nitrate cleaves the entire side-chain to C_{19} steroids. A more generally applicable procedure for the synthesis of steroidal α-keto acids is described in this paper. The principle of the method is based on the observation that α-keto aldehydes undergo an intermolecular dismutation in the presence of small amounts of cyanide ion to produce a mixture of an α-keto-acid and an α-ketol (7). The intermediate cyanohydrin may also utilize an external oxidant such as methylene blue or dichlorophenol-indophenol (7). In either case, the oxidation results in the formation of acid and in the regeneration the cyanide catalyst. This approach was originally applied to the synthesis of pyruvic acid from methylglyoxal (8), but can readily be used for the synthesis of steroid acids. The oxidation of 21-dehydrosteroids of the type IV via the cyanohydrin V at C-21 probably involves the enediol VI which, through the acetonitrile VII, is converted by hydrolysis to the α-keto acid VIII. When methylene blue was used as the oxidant, the reaction was easily followed by noting the fading of blue color. However, the methylene blue partitioned itself between the organic and aqueous phases during extraction of the steroid into solvent. Several washings with dilute acid were needed to remove the dye from the solvent phase. An alternative method was therefore developed using chromium trioxide at


HC = O
C = O
R
R
O
HCN
CN
H—C—OH
C
R
R
O
CN
C
OH
C-OH
R
R
O
IV
V
VI
Oxidant
a, R = R' = H
b, R = OH, R' = H
c, R = H, R' = H, OH
d, R = OH, R' = H, OH
e, R = OH, R' = O
CN
C = O
C = O
R
R
O
H2O
COOH
C = O
R
R
O
VII
VIII
COOH
R
R
O
IX

neutral pH as the oxidizing agent. These procedures have been successfully used to synthesize the 20-keto-21-oic acids corresponding to 11-deoxycorticosterone (VIIIa), corticosterone (VIIIc), and 17α-hydroxy-11-deoxycorticosterone (VIIIb). The syntheses of the 21-dehydro derivatives (IVa-e) have been described (9,10).

That the major products were not the 20-hydroxy-21-oic acids was shown by the formation of bis-2,4-dinitrophenylhydrazones and by the bands in the infrared region at 1670 and 1718-1732 cm^{-1} due respectively to the 3- and 20-ketones. Assignment of frequencies was made difficult because of the carbonyl stretching bands in the same region derived from the carboxyl group. Evidence was obtained that ring D of the acid VIIIb was intact by bismuthate cleavage to the known 4-androstene-3,17-dione, and that ring D of acid VIIIc was intact by periodate oxidation to the etienic acid IXc. The presence of a singlet at 4.45δ in the NMR spectrum of VIIIc provided evidence that the 11β-hydroxy group remained intact during the oxidation of 21-dehydrocorticosterone by methylene blue or chromate ion.

EXPERIMENTAL SECTION

Melting points were determined with an electrically heated block (Fisher-Johns) apparatus and are uncorrected. Ultraviolet spectra were obtained in ethanol solution using a Cary model 15 spectrophotometer. Infrared spectra were run in potassium bromide pellets on the Perkin-Elmer model 221 instrument. Peaks are designated as weak (w), medium (m), or strong (s). Proton magnetic resonance spectra were obtained on a Varian HA-100 spectrometer. The positions of the peaks are reported in ppm downfield from internal tetramethylsilane. Deuteropyridine was used as the solvent throughout. Optical rotations were determined at 589 mμ with a Zeiss polarimeter using a 1 decimeter cell. Nonaqueous titrations were performed in 20% methanol in benzene with sodium methoxide as titrant and using the Radiometer SBR2/SBU1/TTA1 apparatus. For

thin layer chromatography, silica gel (Merck GF_{254}) was used. Crystallized products were dried under vacuum at room temperature over phosphorus pentoxide.

Oxidation of 11β-hydroxy-3,20-diketo-4-pregnene-21-al (21-dehydrocorticosterone) (IVc) to 11β-hydroxy-3,20-diketo-4-pregnene-21-oic acid VIIIc. One gram (2.9 mmoles) of 21-dehydrocorticosterone (11) in 15 ml of acetone was diluted to 45 ml with 0.1 M sodium phosphate, pH 6.8. Potassium cyanide (10 mg, 0.15 mmoles) was added with stirring immediately followed by the dropwise addition of a 1% aqueous solution of methylene blue. As the color faded, more methylene blue was added until the dye was no longer bleached. The solution was concentrated and acidified with hydrochloric acid. The steroid acid was then extracted into sodium bicarbonate (0.1 N) and crystallized from aqueous solution after acidification. Yield was 400 mg, mp 212-216° (dec); λ_{max} (ethanol) 237 mμ (ϵ 14,600); $[\alpha]_D^{27°}$ = +208 (c = 0.5, ethanol); tlc in ethyl acetate: carbon tetrachloride: formic acid (66:33:1), gave a single spot R_f = 0.16; R_f = 0.00 in ethyl acetate: carbon tetrachloride (66:33); molecular weight: calculated 360.43; determined by titrimetry, 360.53; ir (KBr) 3470 (s), 2940 (s), 2600 (w), 2470 (w), 2350 (w), 1718 (s), 1670 (s), 1640 (s), 1632 (s), 1330 (m), 1245 (m), 955 (m), 945 (m), 870 (m). NMR (deuteropyridine) δ 1.347 (18 CH_3), 1.567 (19 CH_3), 4.450 (11β OH), 5.819(4H), 7.802 (21 COOH, disappears in D_2O).

Anal. Calcd for $C_{21}H_{28}O_5$: C, 69.9; H, 7.8. Found: C, 69.6; H, 8.0.

11β-Hydroxy-3,20-diketo-4-pregnene-21-oic acid methyl ester. Twenty mg (55 μmoles) of the free acid VIIIc were quantitatively converted to the methyl ester by reaction with diazomethane. Purity of the product, recrystallized from ethyl ether, was evaluated by tlc in chloroform: ethanol (95:5), and benzene: ethanol (9:1). In each case a single spot, R_f = 0.70 and 0.54, respectively, was obtained. mp 181-183 (dec); ir (KBr) 3490 (s), 3360 (s), 2920 (s), 1730 (s), 1715 (s), 1690 (s), 1670 (s), 1650 (s), 1613 (m), 1272 (m), 1090 (m), 1082 (m), 868 (m).

Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.6; H, 8.1. Found: C, 70.1; H, 8.2.

11β-Hydroxy-3-keto-4-androstene-17β-carboxylic acid IXc. Ten mg of the free acid VIIIc were oxidized with periodic acid as described by Mason et al. (11). The resulting steroid acid was identical with authentic IXc as determined by tlc in ethyl acetate: formic acid (99:1) and by the exact correspondence of infrared spectra; mp, 247-255° (lit. (12) 255-260°).

11β-Hydroxy-3,20-diketo-4-pregnene-21-oic acid-3,20-bis-2,4-dinitrophenylhydrazone. A solution of 10 mg (23 μmoles) of 11β-hydroxy-3,20-diketo-4-pregnene-21-oic acid in methanol was treated

overnight with 25 mg (0.13 mmoles) of 2,4-dinitrophenylhydrazine in 1 ml of 1.1 M hydrochloric acid in 80% methanol. The crystals of the phenylhydrazone were washed with water and methanol and dried. λ_{max} (ethyl acetate: ethanol, 1:10) at pH 3, 383 mμ (ϵ = 554,000); λ_{max} at pH 14, 440 mμ (ϵ = 258,000).

Anal. Calcd for $C_{33}H_{36}O_{11}N_8 \cdot H_2O$: C, 53.5; H, 5.2; N, 15.1. Found: C, 53.4; H, 5.2; N, 15.0.

17α-Hydroxy-3,20-diketo-4-pregnene-21-oic acid (VIIIb). 17α-hydroxy-3,20-diketo-4-pregnene-21-al (1 g, 2.9 mmoles) was oxidized by the method used in the synthesis of VIIIc. Two recrystallizations from aqueous hydrochloric acid gave 250 mg of the acid, mp 171-172°; tlc in ethyl acetate: formic acid (99:1), R_f = 0.32. Purity was 95 to 97%. λ_{max} (ethanol) 238 mμ (ϵ = 14,300); $[\alpha]_D^{27°}$ = +184 (c = 0.5, ethanol); ir (KBr) 3740 (s), 2980 (s), 2920 (s), 2630 (w), 2500 (w), 2375 (w), 1750 (s), 1732 (s), 1660 (s), 1640 (s), 1628 (s), 1370 (m), 1238 (s), 938 (m), 897 (m), 870 (m). NMR (deuteropyridine) δ 1.030 (18 CH_3), 1.047 (19 CH_3), 5.797 (4 H) 7.112 (21 COOH and 17α OH, disappear in D_2O).

17α-Hydroxy-3,20-diketo-4-pregnene-21-oic acid methyl ester. Twenty mg (46 μmoles) of keto acid VIIIb were quantitatively converted to the methyl ester by reaction with diazomethane. Recrystallization of the crude product from methylene chloride and hexane gave 18 mg of the ester, mp 185-188° (dec.); tlc in carbon tetrachloride: ethanol (9:1) showed a single component, R_f = 0.22. ir (KBr) 3440 (s), 2950 (s), 2920 (s), 1732 (s), 1710 (s), 1650 (s), 1600 (m), 1235 (s), 1045 (s), 957 (m), 870 (m).

Anal. Calcd for $C_{22}H_{30}O_5 \cdot 0.5C_6H_{14}$: C, 71.9; H, 8.9. Found: C, 72.2, H, 9.2.

17α-Hydroxy-3-keto-4-androstene-17β-carboxylic acid (IXb). 17α-Hydroxy-3,20-diketo-4-pregnene-21-oic acid (10 mg, 23 μmoles) was oxidized with periodic acid as described (11). The resulting product (4 mg, recrystallized from ether) was identical to an authentic sample of IXb on the basis of mp, 238-242° (lit. (13) 232-240°), chromatographic mobility on tlc in ethyl acetate: formic acid (99:1) and correspondence of ir spectra.

Oxidation of VIIIb with sodium bismuthate. 17α-Hydroxy-3, 20-diketo-4-pregnene-21-oic acid (10 mg, 23 μmoles) was shaken overnight with 25 mg solid sodium bismuthate in 1 ml 50% aqueous acetic acid. The resulting oxidation product had chromatographic mobility in methylene chloride: ethyl acetate (9:1) and chloroform, and infrared spectrum identical with that of 4-androstene-3,17-dione, but was not otherwise characterized.

17α-Hydroxy-3,20-diketo-4-pregnene-21-oic acid 3,20-bis-2, 4-dinitrophenylhydrazone. A solution of 10 mg (23 μmoles) of VIIIb

in methanol was reacted overnight with 25 mg (130 μmoles) of 2,4-dinitrophenylhydrazine in 1 ml of 1.1 M hydrochloric acid in 80% methanol. The crystals of the phenylhydrazone were washed with water and methanol and dried. λ_{max} (ethanol: ethyl acetate, 10:1) at pH 3, 379 mμ (ϵ = 461,000); λ_{max} at pH 14, 440 mμ (ϵ = 333,000).

Anal. Calcd for $C_{33}H_{36}O_{11}N_8 \cdot H_2O$: C, 53.5; H, 5.2; N, 15.1. Found: C, 54.2; H, 5.2; N, 15.0.

Oxidation of 3,20-diketo-4-pregnene-21-al to 3,20-diketo-4-pregnene-21-oic acid (VIIIa) with methylene blue. 3,20-Diketo-4-pregnene-21-al (IVa) (1 g, 3.0 mmoles) was oxidized by the method used in the synthesis of VIIIc. Yield was 200 mg.

Anal. Calcd for $C_{21}H_{28}O_5$: C, 73.3; H, 8.19. Found: C, 73.1; H, 8.38. λ_{max} (ethanol) 240 mμ (ϵ 14,300).

Oxidation of 3,20-diketo-4-pregnene-21-al to 3,20-diketo-4-pregnene-21-oic acid (VIIIa) with chromium trioxide. To a solution of 100 mg (1.0 mmole) of chromium trioxide and 5 mg (0.075 mmoles) of sodium cyanide in 250 ml of 0.05 M N-tris-(hydroxymethyl)-methylglycine (Tricine) at pH 7.2 was added dropwise and with stirring 250 mg (0.76 mmoles) of 3,20-diketo-4-pregnene-21-al dissolved in a minimum amount of methanol. After 30 min, 0.1 ml of formaldehyde was added, and 10 min later the solution was acidified to Congo red paper with concentrated hydrochloric acid. The steroid acid was then extracted into sodium bicarbonate (0.1 N) and crystallized from aqueous solution after acidification. Yield was 159 mg, mp 181-184° (dec) (lit. (6) 183-186°); λ_{max} (ethanol) 241 mμ (ϵ 16,700); $[\alpha]_D^{27}$ = +211 (C = 0.5, ethanol) (lit. (6), + 173); tlc in ethyl acetate: formic acid (99:1) gave a single spot, R_f = 0.44; ethyl acetate, R_f = 0.00. ir (KBr) 3450 (mw), 2930 (s), 2580 (w), 2480 (w), 2310 (w), 1712 (s), 1622 (s), 1330 (w), 1265 (m), 1238 (m), 953 (m), 870 (m). NMR (deuteropyridine) δ 0.808 (18 CH_3), 1.017 (19 CH_3), 5.945 (4H).

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.2; H, 8.2. Found: C, 73.0; H, 8.3.

3,20-Diketo-4-pregnene-21-oic acid methyl ester. Fifteen mg (43.5 μmoles) of the free acid VIIIa were quantitatively converted to the methyl ester by reaction with diazomethane. Purity of the product recrystallized from aqueous ethanol was evaluated by tlc. A single spot appeared in benzene: ethanol (90:10), R_f = 0.82; carbon tetrachloride: ethanol (90:10), R_f = 0.66; ethyl acetate (water sat'd), R_f = 0.93. mp 108-109° (recrystallized to constant value from ethanol-water); ir (KBr) 3400 (mw), 2920 (s), 1730 (s), 1715 (s), 1660 (s), 1605 (m), 1265 (s), 1155 (s), 1050 (m), 952 (m), 860 (m).

Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.7; H, 8.4. Found: C, 73.9; H, 8.6.

3,20-Diketo-4-pregnene-21-oic acid-3,20-bis-2,4-dinitrophenylhydrazone. A solution of 10 mg (29 μmoles) of 3,20-diketo-4-pregnene-21-oic acid in methanol was treated overnight with 25 mg (0.130 mmoles) of 2,4-dinitrophenylhydrazine in 1 ml of 1.1 M hydrochloric acid in 80% methanol. The crystals of the phenylhydrazone were washed with water and methanol and dried.

Anal. Calcd for $C_{33}H_{36}O_{10}N_8$: C, 56.2; H, 5.15; N, 15.9. Found: C, 56.2; H, 5.2; N, 15.8.

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